STRUCTURAL RELATIONSHIP OF MYOSIN ISOENZYMES

Proteolytic digestion patterns of heavy chain components from fast muscles, and comparison with other muscle types

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1. Introduction

Muscle myosins are made up in all known cases of one pair of heavy chains and two pairs of light chains, the so-called alkali and regulatory subunits. Muscle type specificity of myosin has been ascribed to differences in the light and also the heavy subunits. Indeed, the heavy chains of myosins from slow, fast, and cardiac muscles have been reported to differ immunologically [1], and the last two also chemically [2]; these slow, fast and cardiac myosins are known to display different ATPase activities.

Electrophoresis of myosin in its native state has demonstrated that in each of these muscles it is present in several isoenzymic forms [3,4]. In the case for instance of the fast-twitch muscles, such as chicken pectoralis and posterior latissimus dorsi or rabbit skeletal muscle, three myosin populations are found, which differ in their contents of the two alkali light chains, the subunits A_1 and A_2. They contain, respectively, two A_1, two A_2, and one of each light chain per molecule of myosin, and correspond therefore to A_1 and A_2 homodimers and the A_1 A_2 heterodimer [4,5].

To determine whether the differences between these myosin components are confined to their alkali light chains, or extend also to the heavy chains, we have carried out a comparative study of the proteolytic digestion patterns of the latter from all the three fast muscle isoenzymes. After electrophoretic separation in non-dissociating conditions, the heavy chains of each resolved isoenzyme were separated from the light chains and recovered by way of a second electrophoresis in the presence of sodium dodecyl sulphate (SDS); proteolysis of the protein in the slice of gel, according to Cleveland et al. [6], was then performed and the products separated by a third electrophoresis. The heavy chains of the fast muscles' myosin isoenzymes were found to give rise to identical digestion patterns; within the limits of the experimental method of detection used, this implies that the heavy chains of the three isoenzymes present a high degree of similitude. By contrast, the myosins from slow-tonic and ventricular muscles exhibited marked differences in digestion patterns, showing that the heavy chains of myosins from different types of muscles are indeed distinguishable species.

2. Experimental

2.1. *Materials*

Myosins were extracted from chicken pectoralis, posterior and anterior latissimi dorsi muscles and from rat cardiac ventricles, with 3 vol. Guba-Straub buffer. *Staphylococcus aureus* V8 protease was obtained from Miles Lab. Molecular weight protein markers were from Pierce.

2.2. *Electrophoretic techniques*

Electrophoresis in non-dissociating conditions, which led at the same time to purification of the myosin and separation into isoenzymes was done in 0.04 M sodium pyrophosphate (pH 8.5), 1 mM EDTA and 0.01% 2-mercaptoethanol [4]. Cylindrical gels (6 × 0.5 cm), 3.2% in acrylamide and 0.115% in

methylenebisacrylamide were used. Electrophoresis was performed at 2°C at constant 50 V for 24 h. After rapid staining and destaining (~15 min total), the zones corresponding to each myosin isoenzyme were cut from the gels and soaked in 0.0625 M Tris–HCl (pH 6.8), 1% SDS, 1% 2-mercaptoethanol and 10% glycerol; after blotting, the slices of gel were kept frozen at –20°C. Each myosin band contained 1.5–2 μg protein; 32 of these bands were used for 1 proteolysis experiment.

The second electrophoresis was done in the presence of SDS according to [7]. It allowed the isolation of pure heavy chains at high concentration by stacking in a single zone the protein contained in 16 identical gel slices. Cylindrical tubes (11 × 0.6 cm) were used; 3 cm separating and 2.5 cm stacking gels were, respectively, 5% and 3% in acrylamide. Electrophoresis proceeded at 3 mA/gel for 3 h. After the run, the heavy chain zones were stained, cut out and kept as above; the equilibrating buffer was 0.125 M Tris–HCl (pH 6.8), 0.1% SDS, 1 mM EDTA and 1 mM dithiothreitol.

Finally, the proteolysis of the heavy chains and its analysis by SDS–gel electrophoresis were performed according to [6]. 7 cm separating and 2 cm stacking gels, respectively, 12% and 5% in acrylamide were used in cylindrical tubes (11 × 0.6 cm). Two of the previous slices of gel, each containing about 25 μg heavy chains, were placed on top of the stacking gel; the electrophoresis was allowed to proceed at 3 mA/tube for 90 min until the marker dye reached the separating gel. *Staphylococcus* protease (2 μg) (10 μl 0.2 mg/ml solution in 0.125 M Tris–HCl (pH 6.8), 0.1% SDS, 1 mM EDTA and 20% glycerol) was then applied and the run was continued at the same current for 4.5 h.

Myosin ATPase activity was determined in the non-dissociating electrophoretic conditions described above, by replacing the pyrophosphate with ATP, incubating the gel with $CaCl_2$ and observing the appearance of a white precipitate of calcium phosphate [3].

Densitometric scanning of the gels was performed in a Vernon PH 16 instrument.

3. Results and discussion

The three fast-twitch myosin isoenzymes from chicken posterior latissimus dorsi muscle as resolved by gel electrophoresis in pyrophosphate buffer and stained either for protein or for ATPase activity can be seen in fig.1. By contrast, the rat cardiac ventricle and the chicken slow-tonic anterior latissimus dorsi muscle are shown to contain one main myosin species (in both muscles, depending on the age of the animal, other isoenzymes can be found present [3,4,8]). Figure 1 also shows that the three fast myosin isoenzymes display similar specific activities, which are about 1.3- and 3-times higher than those of cardiac and slow myosins.

Typical densitometric scans of the proteolytic profiles of the heavy chains of each of the three fast myosin isoenzymes, the two homodimers and the heterodimer, are shown in fig.2. They are seen to be indistinguishable within the experimental precision. Identical results were obtained with the isoenzymes

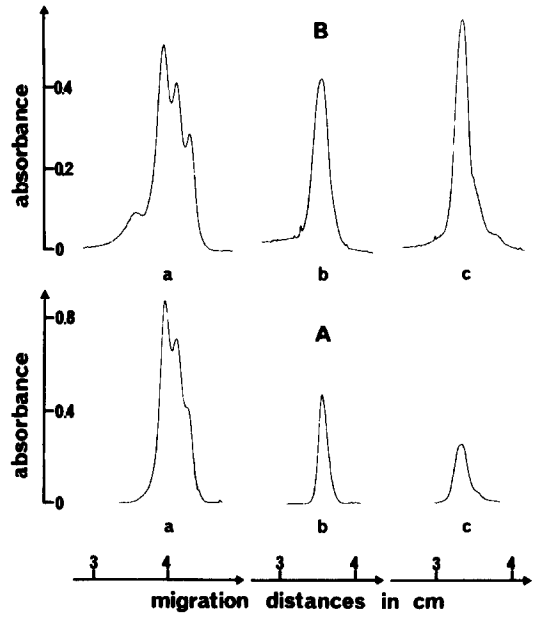

Fig.1. Densitometric scans of the gel electrophoresis patterns of native myosins extracted from: (a) chicken posterior latissimus dorsi muscle, by order of increasing mobility, A_1 homodimer, A_1A_2 heterodimer and A_2 homodimer; (b) rat cardiac ventricle; (c) chicken anterior latissimus dorsi muscle. The same gels were stained first for ATPase and then for protein. (A) Calcium phosphate ATPase staining after 70 min incubation in the activity medium. (B) Coomassie blue protein staining.

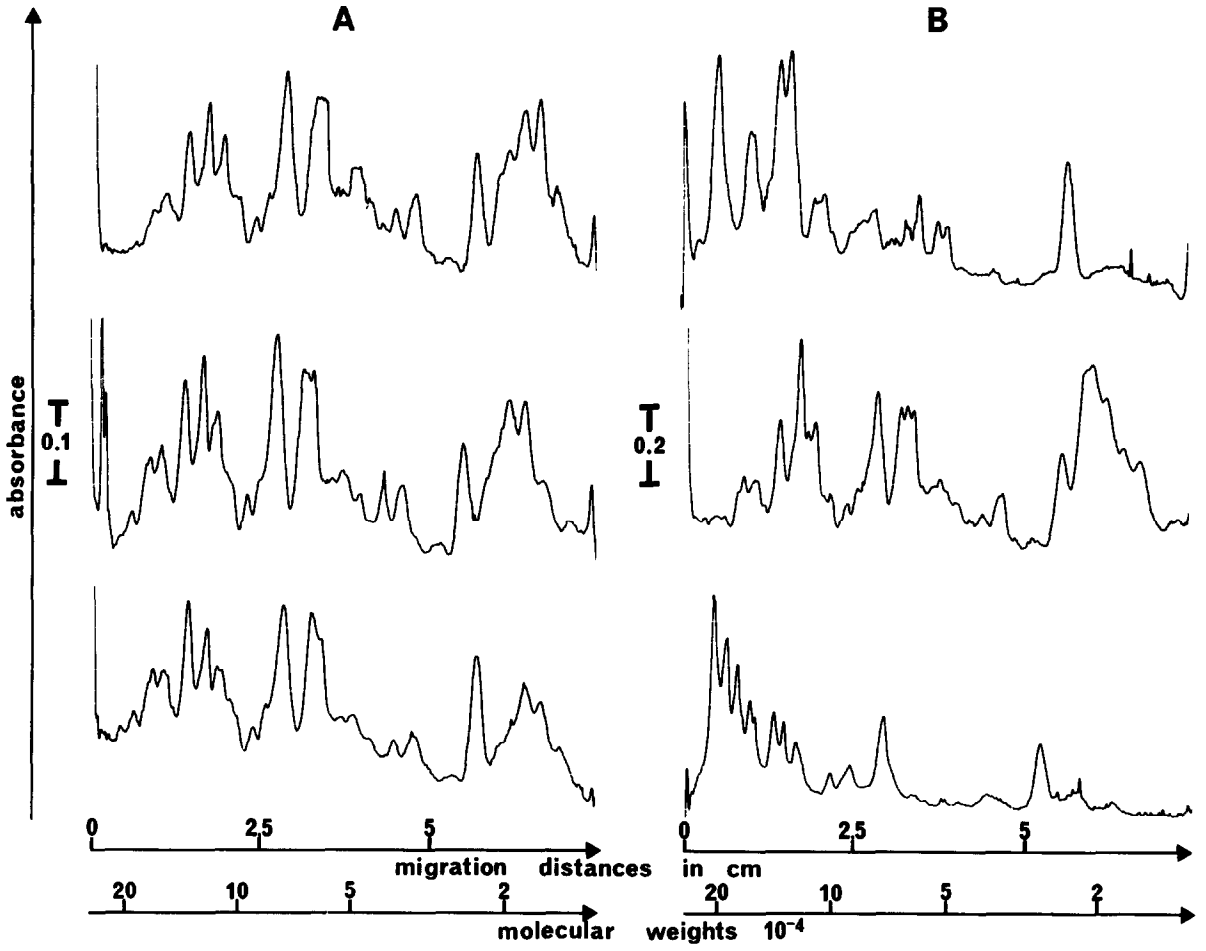

Fig.2. Densitometric scans of the proteolytic patterns of pure heavy chains from different myosins. (A) Fast-twitch posterior latissimus dorsi myosin isoenzymes. From top to bottom: A_1 homodimer; A_1A_2 heterodimer; A_2 homodimer. (B) From top to the bottom: chicken slow-tonic anterior latissimus dorsi myosin; chicken fast-twitch posterior latissimus dorsi myosin; rat cardiac ventricular myosin. Molecular weights are based on protein markers.

from another fast-twitch muscle, chicken pectoralis. For comparison, fig.2 also shows the corresponding profiles of the myosin heavy chains from posterior and anterior latissimi dorsi muscles and from cardiac ventricle. We note first that, as expected from the above results, the mixture of isoenzymes present in fast muscles gives rise, within the limits of experimental reproducibility, to the same proteolytic pattern as each individual isoenzyme. By contrast, myosin heavy chains from fast-twitch, slow-tonic and ventricular cardiac muscles show obvious differences in their proteolytic patterns. Differences between the proteolytic patterns of myosins from a series of muscular and non-muscular sources were observed in solution studies, on mixtures of heavy and light chains [9–11].

From the results presented here, it would be premature to conclude that the three isoenzymes present in fast muscles have identical heavy chains. However, any differences, if they exist, must be of limited extent, and certainly much smaller than those between the heavy chains of myosins from other types of muscles. Some microheterogeneity in the amino acid sequence of fast myosin has indeed

been observed [12,13]; these authors have suggested that at least two kinds of heavy chains are present in fast muscles. However according to [13], 'there appears to be no selectivity in the interaction between individual alkali light chains and these two different heavy chains'. It appears therefore that the electrophoretic mobility differences between the three isoenzymes in non-dissociating conditions (fig.1) depend mainly if not solely on the nature of their alkali light chains. In skeletal fast myosin [14], A_1 does indeed contain an excess of positive charges over A_2, and together with the unusual sequence of its additional segment of chain, this could account for the lower mobility in the pyrophosphate system of the A_1 relative to the A_2 homodimer, the A_1A_2 heterodimer occupying an intermediate position. On this basis, the heavy chains do not contribute to the differences in mobility between the three native isoenzymes; this is consistent with our observation [4] that in a solvent containing phenol, acetic acid and urea, the electrophoretic mobilities of the heavy chains of all three isoenzymes were identical. In contrast, the heavy chains of myosins from other types of muscles, again in accordance with the results of proteolytic fragmentation described above, displayed different mobilities.

Myosins from different types of muscles and within single muscles have been shown to differ in their electrophoretic mobilities in the native state [3,4,15]. The present study allows us to discriminate between the contributions of the heavy and the light subunits to these differences. Both the heavy and the alkali chains are responsible for the differences prevailing between fast-twitch muscle myosins on the one hand, and those of slow-tonic and ventricular muscles on the other. In these last two cases, the differences are evidently a function of the heavy chains alone. In the case of single muscles, while ventricular isoenzymes have been shown [16] to differ only in their heavy subunits, the three fast myosin isoenzymes differ on the contrary mainly or entirely in their alkali light chains.

These observations support the conclusions drawn from the elegant hybridization experiments in [17], namely that the basic ATPase activity of myosin is governed by the identity of the heavy chains; indeed not only the myosins from slow-tonic and cardiac muscles, but also the rat ventricular isoenzymes [8], display different activities, whereas the three fast-twitch alkali light chain isoenzymes are to a first approximation equally active (fig.1). In conclusion, we may note the remark in [18], that the isolated heavy chain of an Acanthamoeba myosin contains full enzymatic activity.

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